



Isolation and Identification of Dye Degrading Bacteria from the Textile Sludge (Soil) Sample

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Isolation and Identification of Dye Degrading Bacteria from the Textile Sludge (Soil) Sample

A Thesis submitted in partial fulfillment of the requirement for the degree
requirement for the degree of Bachelor of Science in Microbiology of BRAC
University

By

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DECLARATION

This is to certify that the research work entitled “**Isolation and Identification of Dye Degrading Bacteria from the Textile Sludge (Soil) Sample**” is submitted by Chinmay Pramanik (10326002) to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology. The content of this thesis have not been submitted elsewhere for the award of any degree or any other publication. I hereby declare that this thesis is my original work based on the results I found. The materials of work found by other researchers and the sources are properly acknowledge and mentioned by reference. I carried out my work under the supervision of Dr. Mohammad Sorowar Hossain.

Dated: August 28, 2014

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FINAL READING APPROVAL

Thesis Title: Isolation and Identification of Dye Degrading Bacteria from the
Textile Sludge (Soil) Sample

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The final form of the thesis report is read and approved by Dr. Mohammad Sorowar Hossain. Its format, citations, and bibliographic style are consistent and acceptable. Its illustrative materials including figures, tables, and charts are in place. The final manuscript is satisfactory and is ready for submission to the Department of Mathematics and Natural Sciences, BRAC University.

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Abstract

Textile industry is one amongst the most chemically intensive industries on the face of the earth and the major contaminator of potable water. It produces huge amounts of different complex chemical substances as a part of unused materials that includes dye in the form of wastewater throughout various stages of textile processing. The direct discharge of this wastewater into surroundings affects its ecological status by inflicting various undesirable changes. Bacteria isolated from the textile sludge (soil) sample and inoculated into screening media containing different types of textile dye in aerobic condition to attain the biodegradability of the dye. Within 24 hours of incubation, this strain proved to be a good decolorizer of the dye. Most preferable pH and temperature for dye degradation were pH 7-7.8 and 29-32 °c under aerobic condition. Various nutrient sources such as sodium chloride, beef extract, yeast extract, or peptone strongly boost the decolorization / degradation process and the bacterial efficiency. All the samples showed positive results for the decolorization of the dyes. Under aerobic condition, color of all the dyes reduced by the bacterial biomass that leads to biodegradation.

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List of Abbreviations

⁰ C	:	Degree Celsius
min	:	Minute
sec	:	Second
h	:	Hour
mg	:	Milligram
gm	:	Gram
L	:	Liter
ml	:	Milliliter
mm	:	Millimeter
nm	:	Nanometer
OD	:	Optical density
e.g.	:	For example
<i>et al.</i>	:	And others
pH	:	Negative logarithm of hydrogen ion concentration
%	:	Percentage
rpm	:	Rotation per minute
UV	:	Ultra violet
spp.	:	Species
NA	:	Nutrient agar

1. Introduction and literature review

1.1 Introduction

The first human made synthetic dye, mauvein, was discovered in 1856 that took over the natural dye quickly. Since then, over 100000 dyes have been generated worldwide with an annual production of over 7×10^5 metric tones. Synthetic dyes are widely used in textile, paper, food, color photography, paper printing, plastic, cosmetics, pharmaceutical, leather and toy industries (Zollinger, 1987; Carliell *et al.*, 1995). As Bangladesh stands among the leading textile exporting countries, textile industries use large quantity of water in its production processes and highly polluted and toxic waste waters are discharged into sewers and drains without any kind of treatment. The textile dyeing industries generate large amount of effluents, sewage sludge and solid waste materials everyday which are being directly discharged into the surrounding channel, agricultural fields, irrigation channels, surface water and these finally enter in to river.



Fig 1: Unused dyestuff polluting the river

Textile and dyeing industrial effluents may cause alteration of the physical, chemical, and biological properties of aquatic environment by continuous change in temperature, odor, noise, turbidity etc. that is harmful to public health, livestock, wildlife, fish, and other biodiversity. The presence of dyes in surface and subsurface water is making them not

only aesthetically objectionable but also causes many water borne diseases, viz. mucous membrane, dermatitis, perforation of nasal septum and severe irritation of respiratory tract. Contamination to this aquatic system brings serious threat to the overall epidemic and socio-economic pattern inside. Industrial effluents impart a minor fraction of chemical load to the environment; its integrity renders the environmental quality fairly deplorable (M. M. Islam, K. Mahmud 2011). A major environmental hazard present in textile industries is the discharge of untreated effluent to the environment, causing pollution of nearby soil and water.



Fig 2: Color of water changes due to the discharge of unused dyestuff

It has been reported earlier that many organisms capable of dye decolorization at laboratory scale, but there are very few reports available on their advantage in treatment processes. The most widely studied white-rot fungus, in this regard is *Phanerochaete chrysosporium* (Reddy, 1995). Efforts to isolate bacterial culture capable of degrading textile dyes started in the 1970s with reports of a *Bacillus subtilis* (Horitsu *et al.*, 1977). Bacterial isolates from soil and sludge sample belonging to *Bacillus* sp. *Alcaligenes* sp. and *Aeromonas* sp. were found to have high dye decolorization ability (Sharma and Saini, 2004). Cynobacteria like *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* decolorized Acid Red 97 and FF sky Blue dye more than 80% after 26 days (Parikh and Madamwar, 2005). Decolorization of Direct yellow and Erio red dyes by bacterial and actinomycetes were studied by Waffa and Moawad, 2003. Other paper suggested that

Pseudomonas sp. (Kothari, 2002) *Escherichia coli*, sulfate reducing bacteria (Yoo, 2002) are efficient dye decolorizer.

Different types of azo dyes can be degraded by many microorganisms, including bacteria (Zimmerman *et al.*, 1982; Hauget *al.*, 1991; Sani and Banerjee, 1999), fungi (Gold and Alic, 1993; Swamy and Ramsay, 1999; Balan and Monteiro, 2001; Novotny *et al.*, 2001), yeast (Martins *et al.*, 1999), actinomycetes (Zhou and Zimmermann, 1993) and algae (Dileket *al.*, 1999). Most azo dyes are reduced anaerobically to the corresponding amines with cleavage of azo bonds by bacterial azoreductase, but they are difficult to degrade aerobically (Zimmerman *et al.*, 1982; Banat *et al.*, 1996). Moreover, fungal ligninolytic enzyme system (lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase) might also be involved in the bio-oxidation of dyes (Gold and Alic, 1993). However, the low pH requirement (Swamy and Ramsay, 1999) for an optimum activity of the enzymes and the long hydraulic retention time for complete decolorization (Banat *et al.*, 1996; Swamy and Ramsay, 1999) are the disadvantages of using fungi. Additionally, fungi may hinder the growth of other beneficial microorganisms. Therefore the uses of fungal decolorization have been restricted.

1.2 Literature review

1.2.1 Historical background

It was reported that the inefficiency in dyeing processes has resulted in 10-15% of unused dyestuff entering the wastewater directly (Zollinger, 1987; Spadarry *et al.*, 1994). Presence of color in textile effluent gives a clear-cut sign of water being polluted, and release of this highly colored and complex effluent can damage directly the receiving water. Furthermore, it is difficult to degrade the mixtures of the wastewater from textile industry by conventional biological treatment processes, because their ratio of BOD/COD is less than 0.3 (Chun and Yizhong, 1999). In some cases, traditional biological procedures were combined with physical or chemical treatment processes to achieve better decolorization (Vandevivere *et al.*, 1998), but chemical or physical - chemical methods are generally costly, less efficient and of limited applicability, and produce wastes, which are difficult to dispose of.

1.2.2 Development of a biological process

As a viable alternative, biological processes have been developed and received increasing interest due to their cost efficiency, ability to produce less sludge, and environmental benignity (Banat et al., 1996). Therefore, to develop a practical bioprocess for treating dye-containing wastewater is of great significance. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Attempt to degrade this complex compound was made in early 90s. But henceforth the success rate was too low.

1.3 Objective of the study

Generally, the textile waste water contains huge amount of various unused dyestuff. To gain extensive acceptance, the dye degrading bacteria should be able to degrade or decolorize a varied kind of dyes. This part of the study was carried out for isolating and identifying the dye degrading/decolorizing microorganisms from the sludge (soil) sample obtained from the textile industry. This study aimed to isolate the bacterial strain and to develop an effective biological process for treatment of textile effluent containing different types of dye.



Fig 3: Direct discharge of unused dyestuff

2. Materials and method

2.1 Materials

2.1.1 Sample

To study, sludge (soil) samples generated by the dying industry were selected.

2.1.2 Dyes

Four types of dye including Acid Red, Direct Yellow, Acid Blue and Direct Black were used. All the dyes were collected from the local dying industry. The main cause for choosing these four dyes, as because, they are widely used in the different dying industry.

2.1.3 Chemicals

The bacteria isolating procedures and the test procedures later used for each dye were carried out in a screening medium (SM medium). The medium contained the following components (for 1 liter of SM): yeast extract- 10g; NaCl- 5.0 g in 1 liter of distilled water with 100 mg of selected dye (K.-C.Chen *et al.*, 2003). The pH of the medium was adjusted to 7. All other chemical for preparing various media were purchased from Hi-media Pvt. Ltd. Mumbai, SRL India. Later on, a modified version of K.C.Chen *et al.*, (2003) used for decolorization study that contained (g l^{-1}) ; K_2HPO_4 5; KH_2PO_4 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 were added along with the above mentioned components. The pH of the medium was maintained at 7.0.

2.2 Method

2.2.1 Sample collection

Sludge sample were collected from the nearby textile industry. All the samples were collected aseptically and preserved in refrigerator at 4°C and they were tested within 24 hours of collection.



Fig 4: Sludge (soil) sample from the dying industry

2.2.2 Screening of decolorizing microorganisms

Four screening medium containing four dyes were prepared and autoclaved prior to inoculation. The pH of the medium was maintained at 7.0. Then the media containing dyes were divided into eight 500 ml conical flask (four as control and four for inoculation) each containing 300 ml of SM. 5 gm of sludge sample containing bacterial culture were taken into 50 ml of sterile saline solution and mixed thoroughly. 1 ml of samples was transferred into each of four conical flasks aseptically. Then the flasks were incubated in a shaking incubator at 30 °c under stirring at 120 rpm.

2.2.3 Dye assay

In the course of time growth of microorganisms was observed by taking samples every 24 hour. To examine the dye concentration, 3ml of sample were centrifuged at 13000 rpm for 15 min and the absorbance values of supernatants were determined at 500 nm.

2.2.4 Isolation of the Bacteria

Using inoculating loop the sample was streak plated on the nutrient agar plate and kept in the incubator at 37 °c. Following 24 hour incubation period, fresh culture plate were prepared from the isolated bacterial colony and preserved at 4 °c.

3. Result and Discussion

3.1 Result

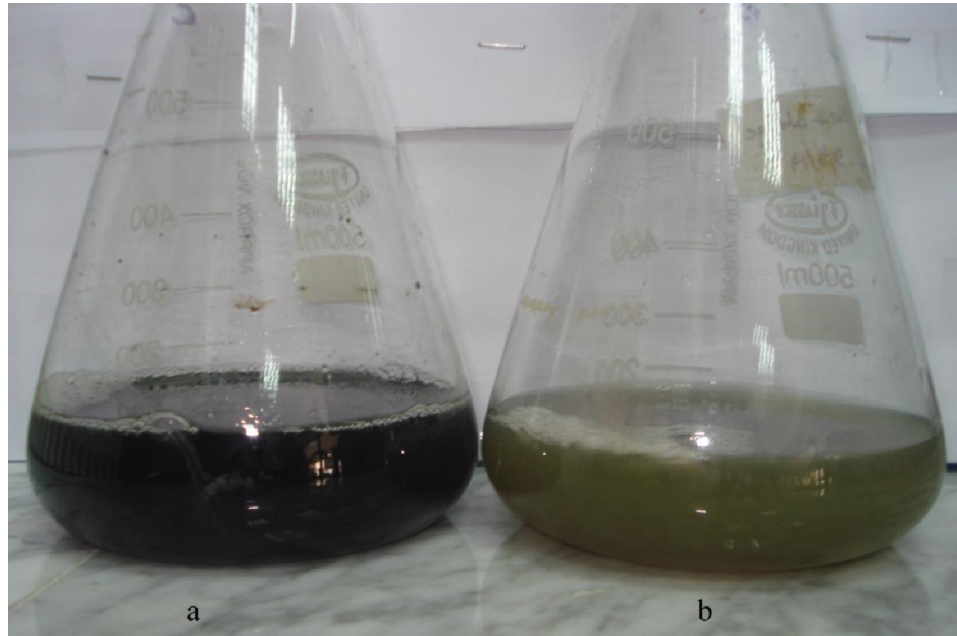


Fig 5: Acid Blue a) control

b) inoculated

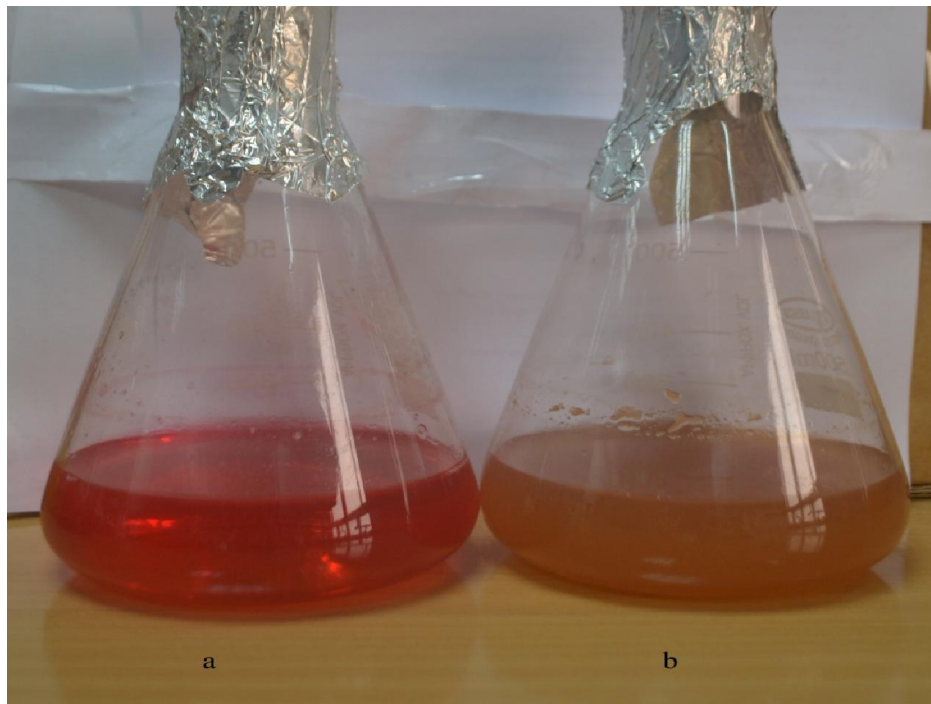


Fig 6: Acid Red a) control

b) inoculated

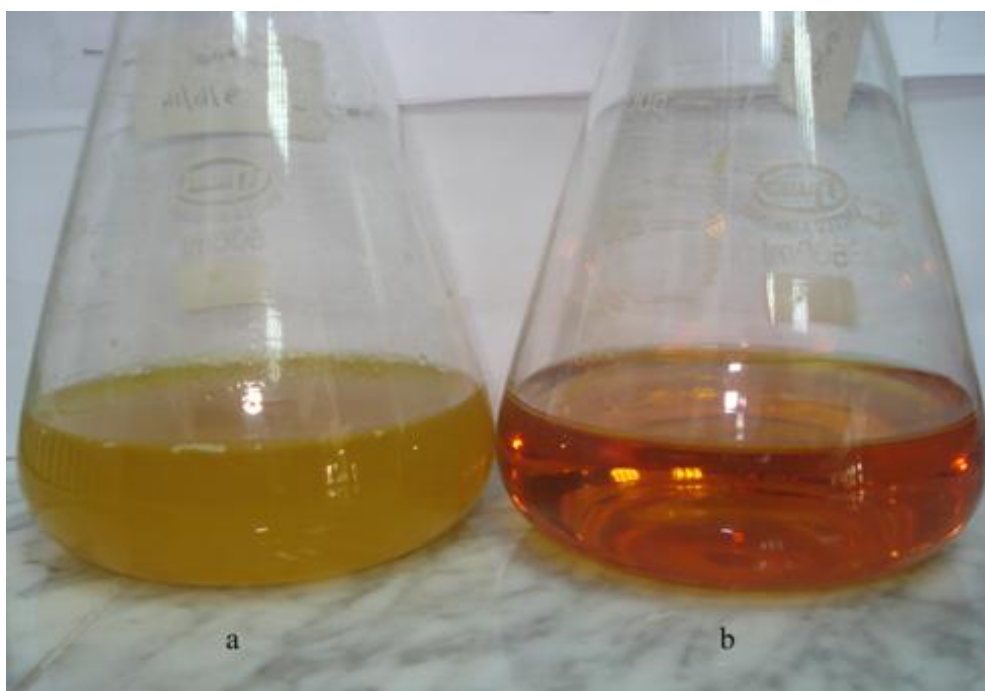


Fig 7: Direct yellow a) inoculated

b) control

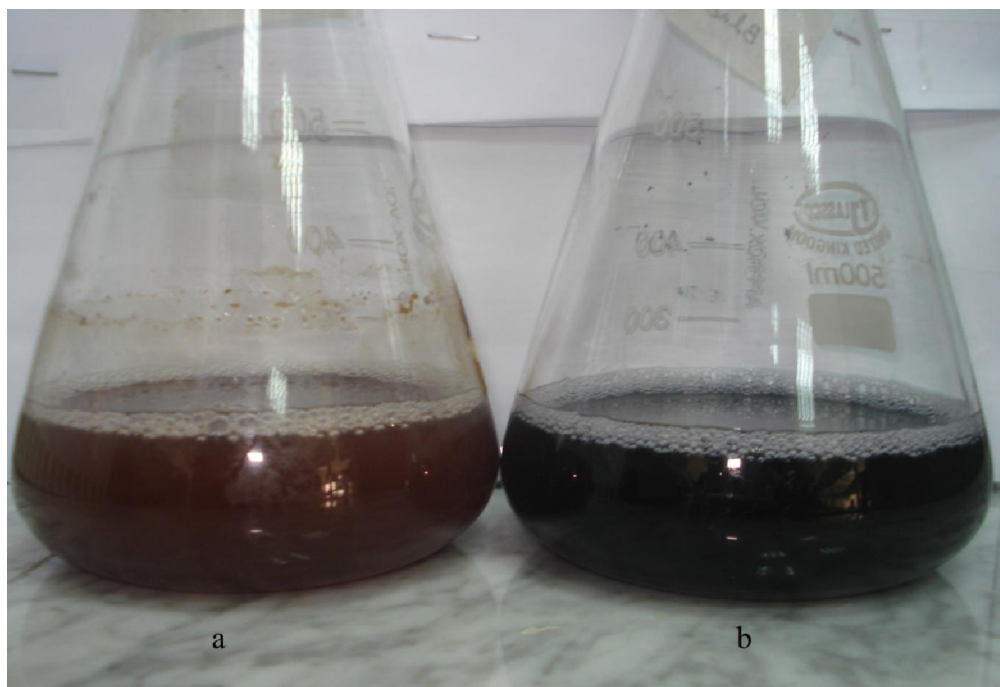


Fig 8: Direct black a) inoculated

b) control

3.2 Graphical representation of color removal

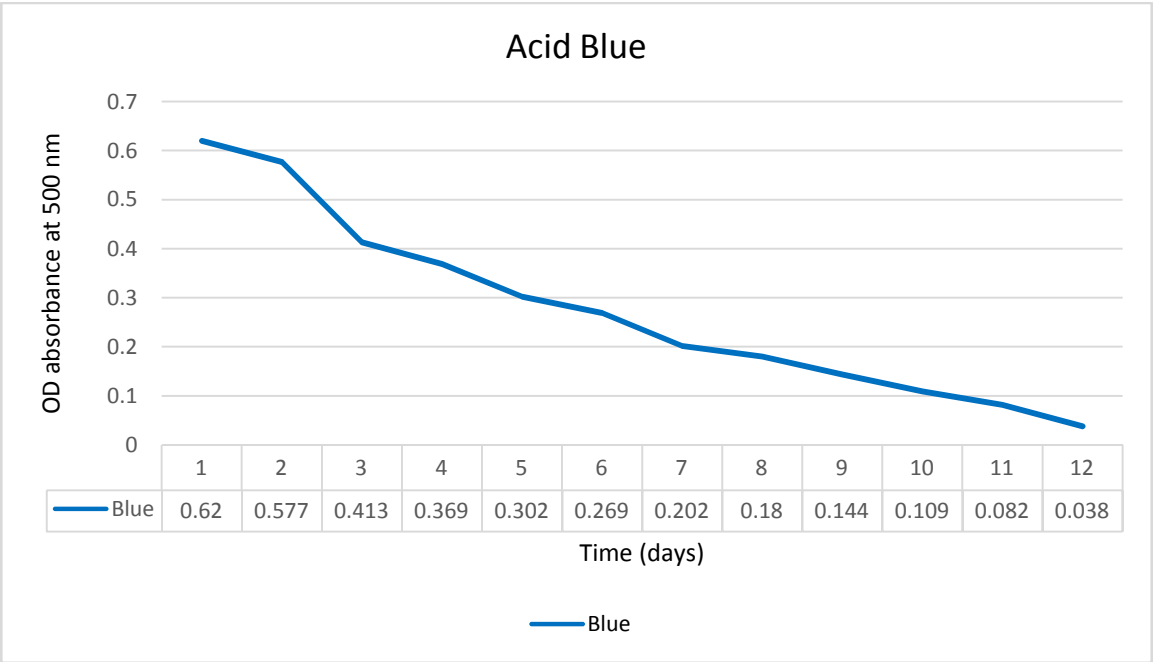


Fig 9: Graphical presentation of Acid Blue degradation

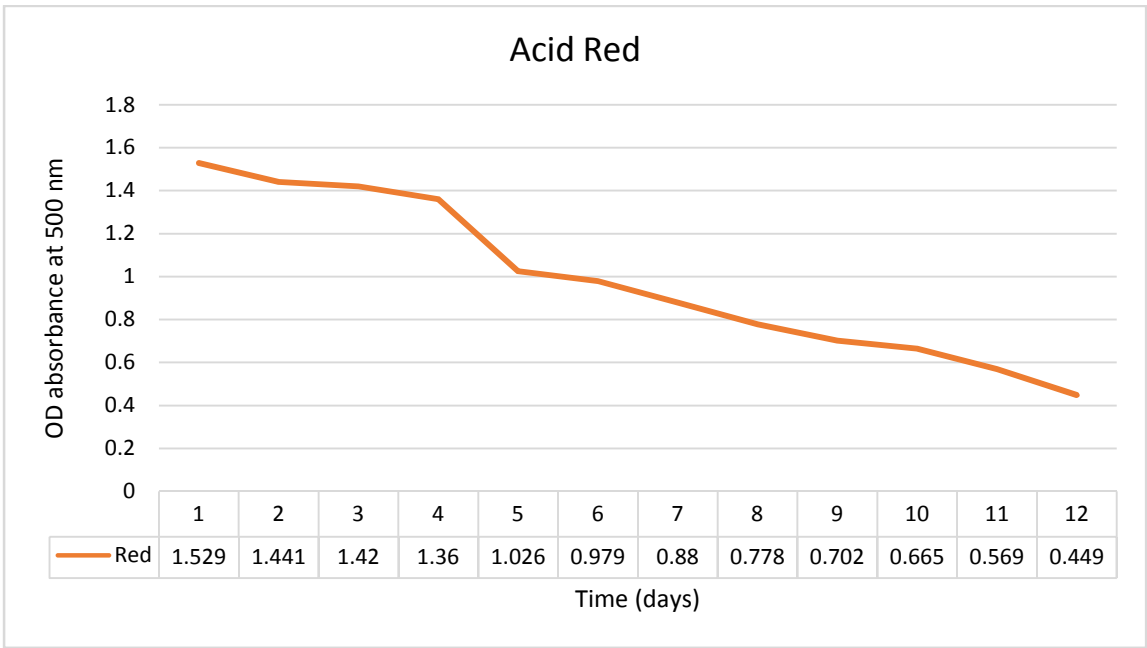


Fig 10: Graphical presentation of Acid Red degradation

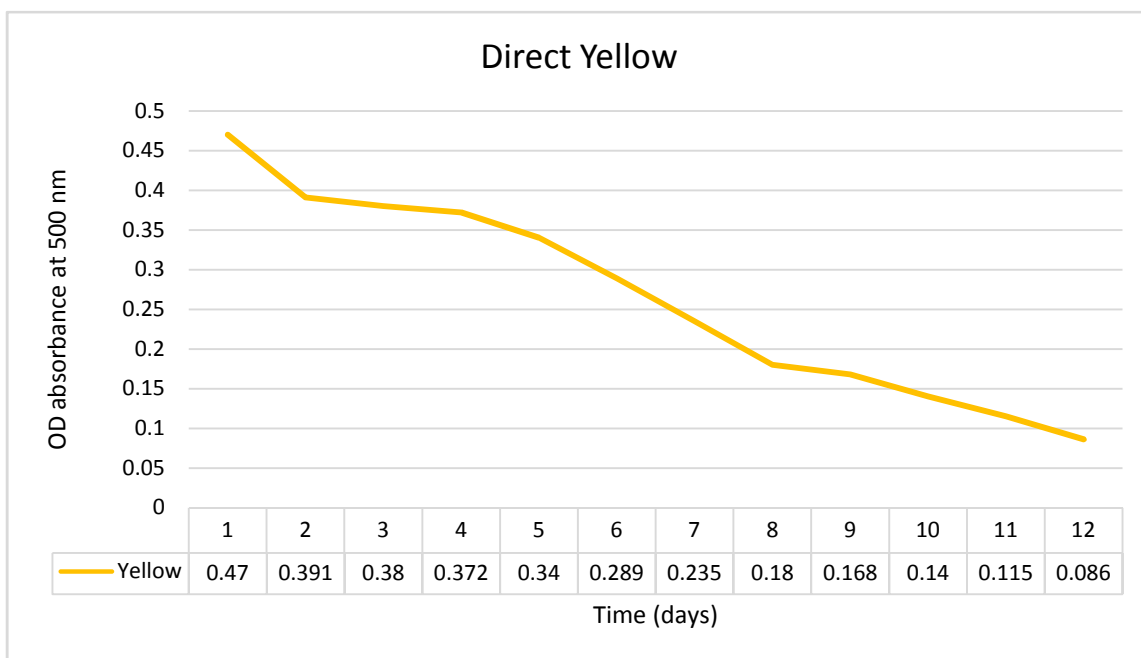


Fig 11: Graphical presentation of Direct Yellow degradation

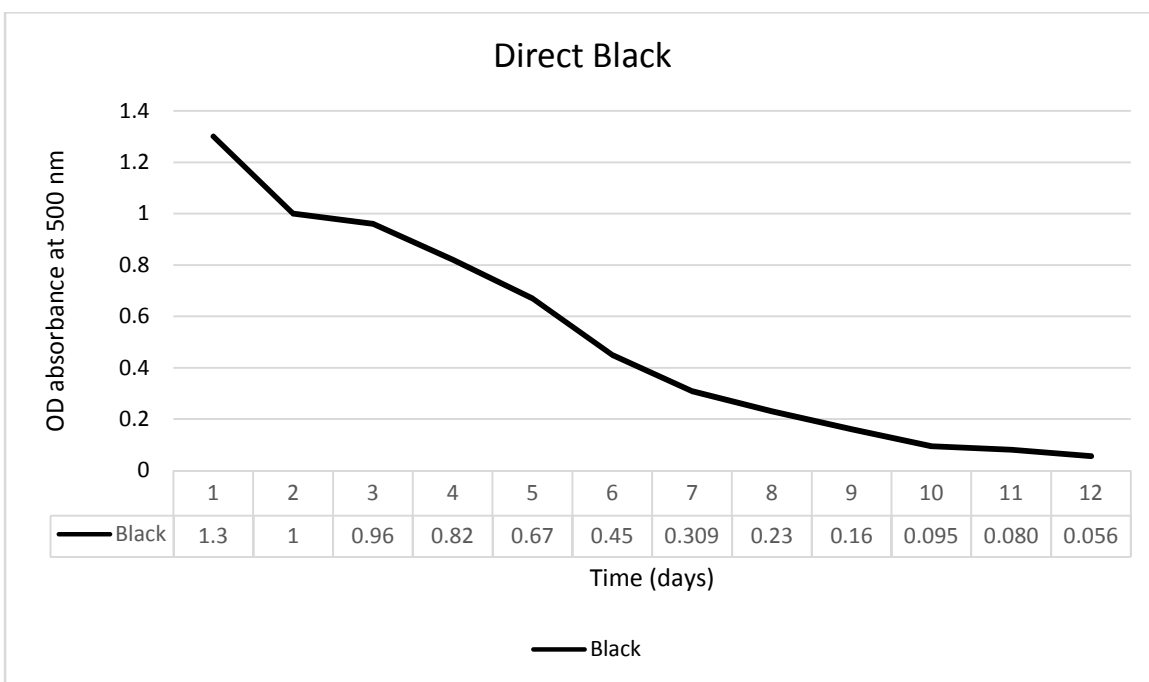


Fig 12: Graphical presentation of Direct Black degradation

3.3 Assay of decolorization

Decolorization activity was expressed in terms of percentage decolorization and was determined by observing the decrease in absorbance at 500 nm of respective dyes. To examine the dye concentration, 3ml of sample were centrifuged at 13000 rpm for 15 min and the absorbance values of supernatants were determined at 500 nm. The degree of decolorization of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV-visible spectrophotometer (1800, Shimadzu, Japan). The decolorization assay was calculated according to the following formula.

$$\text{Decolorization activity (\%)} = (A-B)/A \times 100$$

Where A = initial absorbance

B = Observed absorbance

Dye	Day 2	Day 4	Day 8	Day 12
Direct Yellow	21	21	62	82
Acid Red	6	11	49	71
Acid Blue	7	40	70	94
Direct Black	23	37	82	95

Table 1: color removal (%) based on the OD measured after inoculation.

3.4. Identification of the bacteria

Gram staining was performed and that indicated a Gm + ve long rod shaped organism.



Fig 13: Microscopic view of the bacteria (courtesy- BSMMU lab)

When the organisms were grown on a NA plate, the growth of colonies were moderate with white pigmentation.

Isolates	Gram Staining			Growth Characteristics			
	<i>Gram reaction</i>	<i>Cell type</i>	<i>Cell arrangement</i>	<i>Abundance of Growth</i>	<i>Pigmentation</i>	<i>Opacity</i>	<i>Form</i>
1	Gm +ve	Long rods	Single, double	Moderate	White	Opaque	Filiform

Table 2: Cultural characteristics of the isolate

3.5 Discussion

Dyes of diverse structures are used in the textile industry, and, as a result, the industrial effluents are distinctly variable in composition. In this study, the process made the rate of decolorization very fast and the color removal was almost 50% within 7 days of cultivation, followed by a major change in decolorization for the next 5 days (Fig. 5-8). Fig. 5 and 6 exhibited a slow rate on color removal whereas fig 7 and 8 showed a significant change in color removal. According

to the reports (Knapp and Newby, 1995; Sani and Banerjee, 1999) decolorization of dyes by bacteria can be due to adsorption to microbial cells or to biodegradation.

If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will completely disappear or a new peak will appear (K.-C.Chen *et al.*, 2003). The absorbance peak at 500 nm decreases after 24 h of inoculation and disappeared entirely after 7 days cultivation (Fig. 9-12). As seen in Fig. 9 and 12, there was a significant decline in color intensity or in the peak absorbance at 500 nm. According to the above result, it can be concluded that the color removal from the dye was successful.

4. Conclusion and future works

This paper demonstrated the way to the color removal or dye degradation from industrial wastewater. In conclusion, biodegradation of these types of complex compounds as dyes can be possible. Textile/dying industry with an effective effluent treatment plant produce effluent and sludge that are wealthy source of dye decolorizing/degrading bacterial population. As the dyes are of different in structures and complexity, the percentage of decolorization of different dyes by the same organism are variable. Our result is in very preliminary stage. So, further works needed to be carried for the isolation of effective dye-degrading strains, optimization of these strains in lab, pilot and ultimately in industrial scale.

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APPENDIX-I

Media composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned, all the media were autoclaved at 121°C for 15 min.

1. Yeast agar

Ingredients	Amount (g/L)
Peptic digest of animal tissue	5.000
Yeast extract	3.000
Agar	15.000
Final pH (at25 °c)	7.2±0.2

2. Nutrient agar

Ingredients	Amount (g/L)
Peptic digest of animal tissue	5.000
Meat extract	3.000
Agar	15.000
Final pH (at25 °c)	7.0±0.2

APPENDIX-II

Instruments

The important equipments used through the study are listed below:

❖ Autoclave, Model no: HL-42AE	: Hirayama corp, Japan
❖ Class II Microbiological safety cabinet	: SAARC engineering.
❖ Electric balance, Scout, SC4010	: USA
❖ Refrigerator (4°C)	: Samsung, Korea
❖ Incubator	: Japan
❖ Microcentrifuge, Mikro20	: Germany
❖ Micropipettes	: Eppendorf, Germany
❖ Microwave oven, Model: D90N30 ATP	: Butterfly, China
❖ pH meter, Model no: MP220	: Toledo, Germany
❖ Centrifuge, Model:5804	: Eppendorf, Germany
❖ Spectrophotometer (1800)	: Shimadzu, Japan.